Environmental Health Perspectives
Vol. 44, pp. 189-196, 1982

Cytotoxicity and Ciliostasis in Tracheal Explants Exposed to Cadmium Salts

Michael G. Gabridge* and Rose Ann Meccoli†

Cadmium salts were examined for their biological effects on ciliated respiratory epithelium in hamster tracheal explants. Cadmium chloride and cadmium acetate both caused significant decreases in ciliary motion when tested at 100 μM and above. Reductions in relative ciliary activity were dose-dependent and were first demonstrable at 8-32 hr. The decreased ciliary motion was accompanied by decreases in two key metabolic compounds (ATP and dehydrogenase) which are normally associated with cell viability. Histopathological examination of cadmium-treated tissues showed an epithelium thinner than normal, with extensive vacuolization and few, if any, intact ciliated cells. The various biological effects exerted by cadmium are presented, along with potential mechanisms of pathogenesis for the observed ciliostasis and cytonecrosis. Decreases in adenosine triphosphate appear to play a critical role in the development of cadmium-related effects on cellular function and metabolism.

Introduction

Cadmium is a toxic heavy metal which humans encounter in air, food, water and soil (*1*). Respiratory intake varies and depends primarily on proximity to smelters and factories (*2*, *3*). Such sources can emit up to 15 μg/m^3. In addition, cigarette smokers inhale approximately 2-4 μg/pack (*1*). The effects of cadmium on respiratory tissue include pulmonary edema (*4*), inflammation (*5*) and cytonecrosis of type I alveolar cells (*6*). Levels of lung cell enzymes (e.g., glucose-6-phosphate dehydrogenase) are also increased (*4*).

A brief exposure to a cadmium aerosol can depress clearance of subsequently inhaled pathogens, accompanied with an increase in mortality (*7*). Since cadmium induces ciliostasis in the trachea (*8*), this increased susceptibility to infection could be related to depressed mucociliary clearance. The ciliostatic effect has been studied in detail with tracheal explant techniques. Concentrations of cadmium chloride as low as 1.4 μg/ml caused a significant decrease in ciliary frequency after a 24-hr exposure (*8*), while another study using cadmium acetate reported such changes in as little as 4 hr (*9*).

The current project was designed to examine more closely the *in vitro* effect of cadmium on the ciliated respiratory epithelium. Emphasis was placed on the correlation of ciliostasis and cytotoxicity, the quantitation of cadmium within target tissue, and the relative effects of cadmium chloride and cadmium acetate.

Methods

Tracheal ring explant cultures were prepared from adult male golden hamsters *(Mesocricetus auratus)* with the techniques previously described by Gabridge et al. (*10*, *11*). Basically, the explants consisted of transverse slices of trachea, 1–2 mm thick, the inner surfaces of which were lined with a ciliated epithelium. Cultures were maintained in a thin layer of complete minimal essential medium (MEM; Eagles) with 10% fetal calf serum.

Relative ciliary activity, RCA (*10*, *12*), was calculated during observation of the ciliary motion with 225× phase optics on an inverted microscope. RCA is the product of the percent of the surface

*W. Alton Jones Cell Science Center, Lake Placid, New York 12946.
†Department of Veterinary Pathology, University of Illinois, Urbana, Illinois 61801.

with an intact epithelium (0–100%), and a subjective estimate of vigor of ciliary motion (0–3 +). Biochemical assays of cell viability were based on dehydrogenase activity (primarily succinate, as determined with a tetrazolium chloride reduction assay) (*13*) and ATP content (determined photometrically with a luciferin-luciferase enzyme system) (*14*). For histopathological examination, tracheal explants were rinsed in phosphate buffered saline (PBS, pH 7.4) and fixed in 4% glutaraldehyde in PBS. Specimens were embedded in plastic, sectioned with a glass knife and stained with hematoxylin and eosin.

Solutions of cadmium chloride and cadmium acetate (Mallinckrodt) were prepared in MEM and were filter sterilized with 0.22 μm (mean of diameter pore size) Millipore filter. To determine actual tissue concentrations of cadmium in tracheal explants, neutron activation analysis was employed. Tracheas were rinsed, air dried and stored under vacuum. Samples were heat-sealed in a precleaned polyethylene vial. Known volumes of solution were sealed into similar vials and the sample and standards were sealed in a larger precleaned vial. Vials were irradiated in the rotary sample holder of the University of Illinois Advanced TRIGA Reactor for 8 hr at a flux of 4×10^{12} neutrons/cm^2-sec. After a delay of approximately 7 days, the samples were counted for 2 hr for gamma radiation by using a 10% efficient Ge (Li) spectrometer having a 2.1 keV resolution at the 1332 keV line of ^{60}Co. The activities of the sample and standard, counting times and the standard cadmium concentration were entered into the program of the Nuclear Data 6620 computer to calculate the amount of cadmium in each sample. One standard deviation errors (from unknown uncertainties in sample and standard preparation and from the counting statistics) were propagated to calculate the reported uncertainty in the sample vials.

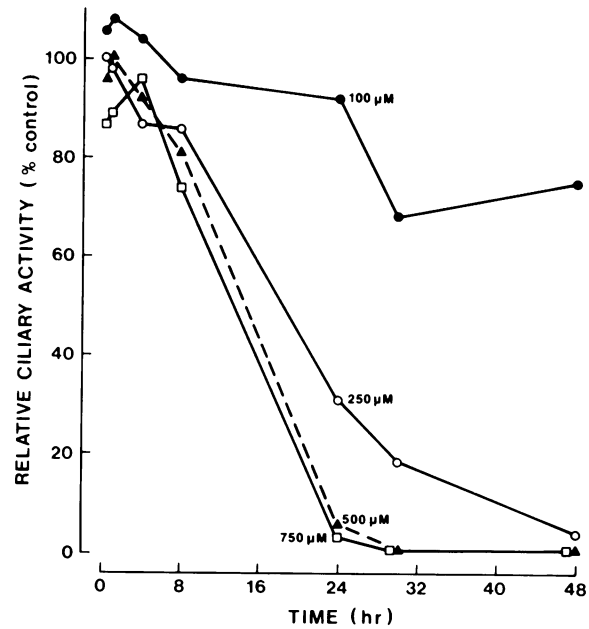

FIGURE 1. Effect of various concentrations of cadmium chloride on ciliary activity in hamster tracheal explants. Mean data from six explants per time point.

Table 1. Effect of cadmium chloride on relative ciliary activity (max = 300) and ATP content of tracheal explants after 24 hr exposure (mean values from six explants).

Concentration, μM	Ciliary activity		ATP content	
	0 hr	24 hr	μg/mg	% of control
0	271	253	1.71	100
100	255	183	1.60	94
250	248	116	0.96	56
500	254	10	0.58	34
750	242	4	0.43	25

Results

Initial studies were conducted in order to determine the effect of cadmium chloride on tracheal explant ciliary motion. The data (Fig. 1) show that cadmium has very definite ciliostatic activity. Doses of 100 to 750 μ*M* caused significant decreases in the percent and relative vigor of the ciliated epithelium, and the effect was dose-dependent (Table 1).

At 100 μ*M*, ciliary motion was nearly normal for the first 8 hr, but then gradually decreased for the next 40 hr. After a total of 48 hr in 100 μ*M* cadmium chloride, ciliary activity was reduced 20%. The drop was much more pronounced in a 250 μ*M* solution. It caused a significant decrease within the first 4 hr. By 48 hr, the 250 μ*M* treatment had reduced ciliary activity to approximately 4% of the control levels. The response to 500 and 750 μ*M* cadmium chloride was even more rapid. A 96% drop in ciliary activity was evident after 24 hr, and by 30 hr the activity was eliminated.

These decreases in ciliary motion were correlated with similar decreases in metabolic activity in cadmium-treated explants. Tracheal explants were incubated in 0, 100, 250, 500 or 750 μ*M* cadmium chloride for 48 hr, scored visually for ciliary motion, and then evaluated for dehydrogenase activity. The data (Fig. 2) show that the percent decrease (relative to MEM control explants) in both ciliary activity and enzyme activity are highly correlated. With the 100 μ*M* concentration, both parameters are between 40 and 70%, while with 250 μ*M* and

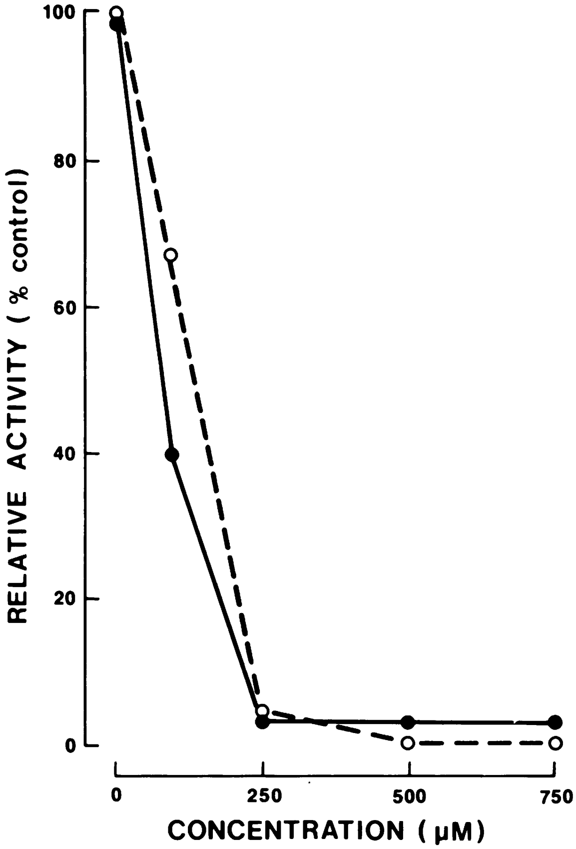

FIGURE 2. Effect of cadmium chloride on (○) relative ciliary activity and (•) dehydrogenase activity of tracheal rings after 48 hr of exposure. Mean data from six explants per time point.

above, the activities range from 0 to 5% after the 48 hr exposure.

A similar, though not as direct, correlation was seen with the ATP content of treated explants. After 24 hr of incubation, the amount of ATP in the tissues was inversely correlated with cadmium dose. Doses of 250 μM caused 50-60% decreases in both ciliary activity and ATP content. Levels of 500 and 750 μM reacted similarly in that they caused reductions in ciliary motion of $\geqslant$ 90% and reductions in ATP of $\geqslant$ 66%. The fact that both the visual measure of ciliary motion and the biochemical assessments of cell viability decreased in dose-dependent fashions, indicates that cadmium chloride induced both ciliostasis and overt cytotoxicity.

In order to determine whether or not these effects were limited to only the chloride salt of cadmium, cadmium acetate was also evaluated in the tracheal explant system. The results (Table 2) show that the effects of cadmium acetate are similar to those of cadmium chloride. A level of 100 μM cadmium acetate caused decreases of 33-43% in ciliary motion and ATP content after 24 hr. The response to 250 μM was even more pronounced, and also included both the function and metabolism of ciliated cells.

The damage induced by cadmium salts (i.e., ciliostasis and cytonecrosis) was also apparent in histological sections of treated tissues (Fig. 3). The lumen of the control tracheal explants was lined with a pseudostratified layer of ciliated epithelial cells. Cilia were common. The ciliated cells and occasional goblet cells composed the layer above the basal layer of undifferentiated cells. When explants were incubated in 250 or 750 μM solutions of cadmium chloride for 24 hr, significant cytonecrosis could be observed.

The ciliated epithelium was largely destroyed by cadmium. Ciliated cells were fewer in number and the epithelium was thin and sparsely populated. Few ciliated tufts on epithelial cells could be detected. Cells tended to round up and lose their tall, columnar type of shape. Vacuolization was common, both around and within the nucleii. Morphological alterations which suggested massive cell destruction were evident at both cadmium concentrations, with almost complete destruction of the epithelium obvious at 750 μM.

In an attempt to quantitate the amount of cadmium actually present in treated tissues, a neutron-activation technique was used. This method was advantageous, in that it requires small quantities of tissue (< 15 mg) and has high accuracy and sensitivity. Our results (Table 3) indicate that levels in excess of 8,000 μg/g were attained in

Table 2. Effect of cadmium acetate on relative ciliary activity and ATP content of tracheal explants (mean data from six explants).

	Ciliary activity				ATP content	
Concentration, μM	0 hr	3 hr	24 hr	% of control[a]	μg/mg	% of control[a]
0	243	238	203	100	2.26	100
100	222	189	115	57	1.52	67
250	239	228	67	33	1.09	48

[a]At 24 hr.

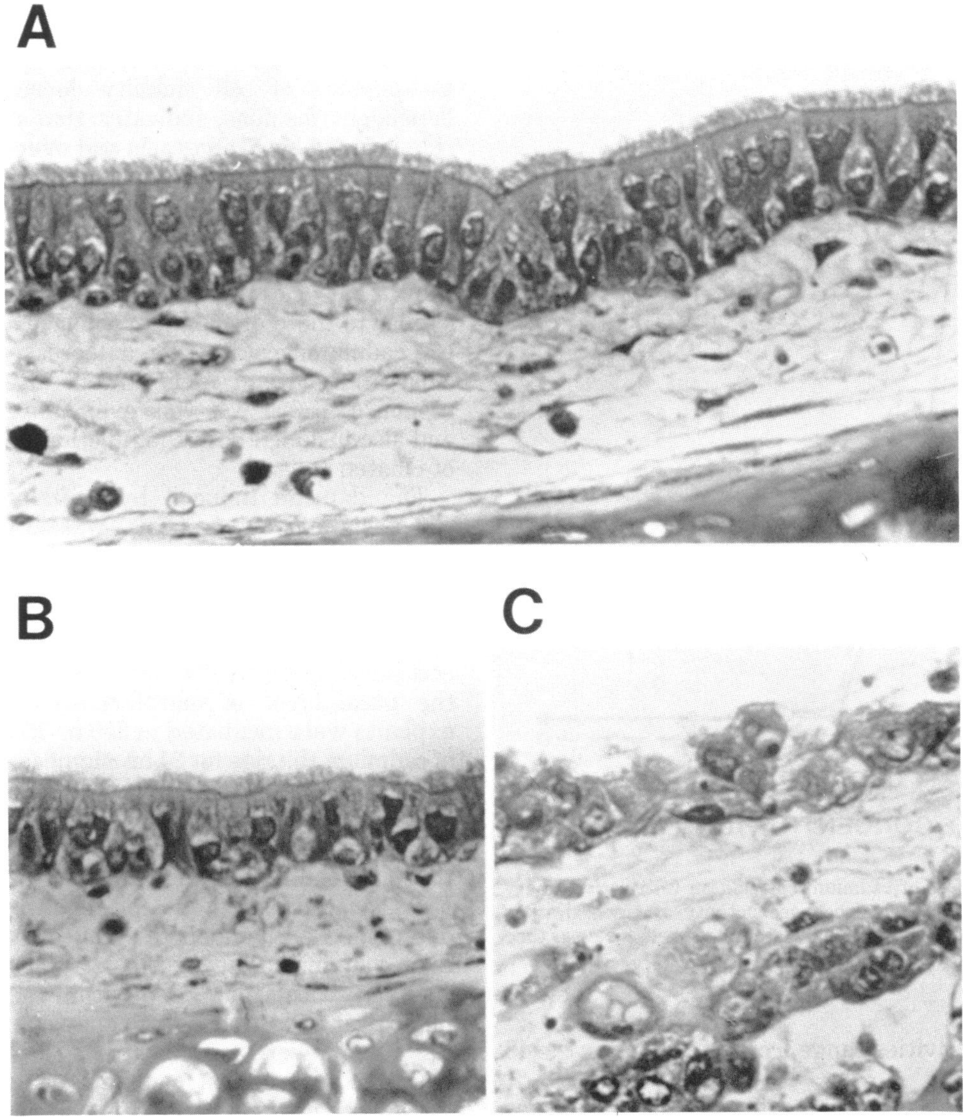

FIGURE 3. Light photomicrographs of control and cadmium-treated hamster tracheal explants after 24 hr: (*A*) MEM control; (*B*) 250 μM $CdCl_2$; (*C*) 750 μM $CdCl_2$. Hemotoxylin and eosin; original magnification 600×.

explants after 24 hr in 750 μM solutions of cadmium chloride. Entry of Cd was time dependent. After 1 hr of incubation, only 785 µg/g could be detected (relative to a base-line level of approximately 30 µg/g). The level increased more than 10-fold during the next 23 hr. These data clearly indicate that elemental cadmium is present in tracheal tissues bathed in cadmium salts.

Discussion

Cadmium is an element which is released into our environment in massive quantities. In a recent review (*11*), Fishbein estimated that the yearly worldwide release of Cd was approximately 5-8 million lb. Of these emissions, 90% come from metal plating and alloy and battery manufacturing. Levels of airborne cadmium particulates in the workplace can be quite high. Values from 110 to 2125 $\mu g/m^3$ were reported by Lauwerys et al. (*15*). For comparison purposes, the levels of cadmium chloride in our current study (100 to 750 μM) ranged from 0.023 to 0.171 g/l.

The *in vivo* effects of cadmium are many and varied. The precise biological response depends on the route of administration, but most organs dis-

Table 3. Time dependence for entry of cadmium (750 μM $CdCl_2$) into tracheal tissue (neutron activation analysis of 40 explants per time sample.

Time, hr	Weight, mg (dry)	Cd content (± SD), µg/g
0	14.33	< 39
1	14.02	785 ± 27
24	13.56	8,560 ± 200

play some degree of cytotoxicity (*4-8, 16-20*). Lung inflammation and necrosis are typically seen in response to aerosols containing 10 mg/m^3, causing a disruption in mucociliary function. This effect has been assessed directly in measurements of ciliary motion (*8, 9*) and indirectly as increased susceptibility to lung infections (*7*).

The significance of these doses and their consequences for the respiratory tract can best be appreciated in a graphic representation of reported air levels of Cd and the various biological effects (Fig. 4). A threshold limit of 200 µg/m^3 was set for industrial workers by the American Conference of Governmental and Industrial Hygienists (*8*). The values in normal air have been reported from several countries, and range from 0.001 to 3 µg/m^3. Factory air levels are much higher, however, and range from 5 to 250 µg/m^3. Levels in this range have been shown to cause ciliostasis, increased infections and inflammation in laboratory animals (*5, 7, 8*). Levels above 1000 µg/m^3 can cause edema and lung cell destruction (*4, 7*). It is apparent that many workers are being exposed to potentially cytotoxic cadmium levels (*15*).

The nature of this cytotoxic response in the ciliated respiratory epithelium was the subject of the current study. We chose to use an *in vitvo* analysis because of the ability to control dosage levels accurately and to monitor biochemical effects. Others have studied the effects of heavy metals in cell cultures of alveolar macrophages (*21*) and lung fibroblasts (*22*), and in explant cultures of rodent trachea (*8, 9*). The latter are an especially appropriate model because the target cells are highly differentiated and are representative of the mucosal surfaces which would be exposed to inhaled cadmium particles. Unfortunately, the data which were available only pertained to ciliary activity, and it could not be ascertained whether the decreased motion was due to ciliostasis and/or to cytotoxicity. The current study examined both effects.

The ciliated tracheal explants which we treated with cadmium chloride and cadmium acetate had significant decreases in ciliary motion, consistent with the observations of Adalis et al. (*8*) and Olsen and Jonsen (*9*). Doses of 100 μM decreased ciliary

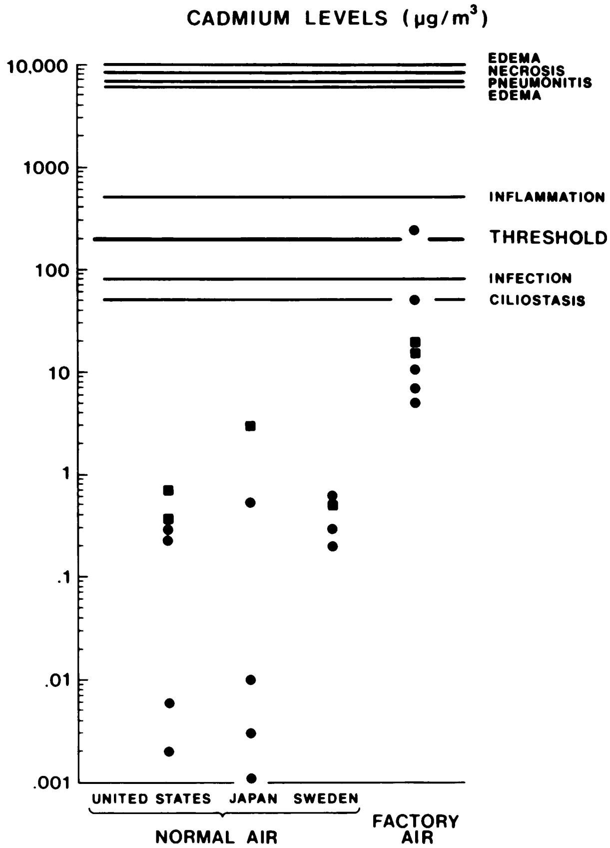

FIGURE 4. Comparison of reported (*1, 3-7*) cadmium levels in air and the amounts required to elicit a biological effect. Squares and circles for each country represent different studies.

activity approximately 20% after 48 hrs. A level of 250 μM acted must faster. Little ciliary vigor, and a markedly reduced area of epithelium with ciliated cell movement, were detectable after 48 hr. The 500 and 750 μM levels nearly eliminated ciliary activity within 24 hr.

This ciliostasis was not merely a consequence of physical interference with ciliary movement (e.g., ciliary strands blocked by excessive mucous production) (*23*), but was a direct result of cell necrosis. Assays for ATP content and dehydrogenase activity indicated that treated tracheal cells were metabolically inactive. The decreased ciliary motion and absence of cell viability were compatible with the histopathological analysis. The morphology of cadmium-treated explants was severely affected. The epithelium was thinned, ciliated cells had been lost and vacuolization of both nucleii and cytoplasm was obvious. These data (parameters of structure, function and metabolism) show that cadmium salts are both ciliostatic and cytotoxic for ciliated respiratory epithelial cells. Levels as low as 100 μM can induce chemically detectable changes in 24-32 hr. The fact that this decreased metabolic activity was actually a reflection of cell destruction was established with electron microscopy (manuscript in preparation).

The mechanism by which these cadmium-related alterations in structure and function occur is not clearly understood. Several related theories have been advanced. Sanders et al. (*24*) suggested that cadmium may alter cellular permeability after binding to membranes, or may enter the cell and bind to sensitive target sites such as sulfhydryl groups. This latter possibility seems plausible in view of the protective effect found when compound WR2721 was administered to rats before they inhaled cadmium oxide aerosols. WR2721 is a thiophosphate which is rapidly converted intracellularly to a substrate with highly reactive sulfhydryl groups.

On the level of an intact organism, other mechanisms may be operative. Corradino (*25*) showed that cadmium did not cause a generalized toxicity, but instead specifically reduced the level of calcium-binding protein. This caused cytotoxic effects related to decreased vitamin D-mediated intestinal calcium adsorption. Other studies (*26, 27*) have illustrated that cadmium can inhibit zinc-dependent enzymes. This is apparently due to competition between cadmium and zinc at cofactor sites on certain enzymes. However, this phenomenon may be species specific, and of more significance in single cell systems (*26*) and laboratory animals than in humans (*27*).

Adalis et al. (*8*) have suggested that cadmium may exert its cytopathology through a diminution of usable ATP. ATP is the energy source for ciliary movement. If ATPase is inhibited by a metallic ion such as cadmium, ciliary activity would be adversely affected. This would be reasonable in light of the data of Sugawara (*28*) and Nechay (*29*). Both investigators demonstrated that cadmium levels as low as 10^{-4} M significantly inhibited ($Na^+ + K^+$) ATPase and (Mg^{2+}) and ($Mg^{2+} + Ca^{2+}$) ATPase in animal tissues.

A prolonged lack of available ATP would also decrease cellular protein and nucleic acid synthesis and reduce the efficiency of the ion pumps necessary to maintain normal intracellular osmotic and ionic pressures (*14, 30*). Our study supports this hypothesis, since it is possible that the measurable ATP losses which we found were a result of all death and thinning of the epithelium after the inactivation of ATPase. Regardless of the precise mechanism, it is clear that cadmium salts can induce cell necrosis and a loss of function which: (a) provides the rationale for the decreased mucociliary clearance and increased mortality from respiratory infections noted in cadmium-treated animals, and (b) underscores the importance for minimizing human exposures to high environmental and occupational cadmium levels.

This work was supported in part by grant HL 26880 from the National Heart, Lung, and Blood Institute. The authors express their gratitude to Dr. P. K. Hopke of the University of Illinois Environmental Research Laboratory for conducting the neutron activation analysis, and to C. Dayton for her editorial review.

REFERENCES

1. Fishbein, L. Environmental metallic carcinogens: an overview of exposure levels. J. Toxicol. Environ. Health 2: 77-109 (1976).
2. Adamsson, E., Piscator, M., and Nogawa, K. Pulmonary and gastrointestinal exposure to cadmium oxide dust in a battery factory. Environ. Health Perspect. 28: 219-222 (1979).
3. Kjellstrom, T., Friberg, L., and Rohnster, B. Mortality and cancer morbidity among cadmium-exposed workers. Environ. Health Perspect. 28: 199-204 (1979).
4. Hayes, J. A., Snider, G. L., and Palmer, K. C. The evolution of biochemical damage in the rat lung after acute cadmium exposure. Am. Rev. Resp. Dis. 113: 121-130 (1976).
5. Bus, J. S., Vinegar, A., and Brooks, S. M. Biochemical and physiologic changes in lungs of rats exposed to a cadmium chloride aerosol. Am. Rev. Resp. Dis. 118: 573-580 (1978).
6. Strauss, R. H., Palmer, K. C., and Hayes, J. A. Acute lung injury induced by cadmium aerosol. I., Evolution of alveolar cell damage. Am. J. Pathol. 84: 561-578 (1976).
7. Gardner, D. E., Miller, F. J., Illing, J. W., and Kirtz, J. M. Alterations in bacterial defense mechanisms of the lung induced by inhalation of cadmium. Bull. Europ. Physiopath. Respir. 13: 157-174 (1977).
8. Adalis, D., Gardner, D. E., Miller, F. J., and Coffin, D. L. Toxic effects of cadmium on ciliary activity using a tracheal ring model system. Environ. Health Res. 13: 111-120 (1977).
9. Olsen, I., and Jonsen, J. Effect of cadmium acetate, copper sulfate, and nickel chloride on organ cultures of mouse trachea. Acta Pharm. Toxicol. 44: 120-127 (1979).
10. Gabridge, M. G., Johnson, C. K., and Cameron, A. M. Cytoxicity of *Mycoplasma pneumoniae* membranes. Infect. Immunol. 10: 1127-1134 (1974).
11. Gabridge, M. G. Hamster trachea organ cultures, Tissue Culture Association Manual I:75-80 (1975).
12. Gabridge, M. G., Barden-Stahl, Y. D., Polisky, R. B., and Engelhardt, J. A. Differences in the attachment of *Mycoplasma pneumoniae* cells and membranes to tracheal epithelium. Infect. Immunol. 16: 766-772 (1977).
13. Gabridge, M. G., and Polisky, R. B. Quantitative reduction of 2,3,4-triphenyltetrazolium chloride by hamster trachea organ cultures: effects of *Mycoplasma pneumoniae* cells and membranes. Infect. Immunol. 13: 84-91 (1976).
14. Gabridge, M. G. and Polisky, R. B. Intracellular levels of adenosine triphosphate in hamster trachea organ cultures exposed to *Mycoplasma pneumoniae* cells or membranes. In Vitro 13: 510-516 (1977).
15. Lauwerys, R., Roels, H., Buchet, J. P., Bernard, A., and Goret, A. Significance of cadmium concentration in blood and urine in workers exposed to cadmium. Environ. Res. 20: 375-391 (1979).
16. Sugawara, C., and Sugawara, N. Effect of cadmium on

vitamin A metabolism. Toxicol. Appl. Pharmacol. 46: 19-27 (1978).

17. Kerkvleit, N. I., Koller, L. D., Baecher, L. G., and Brauner, J. A. Effect of cadmium exposure on primary tumor growth and cell-mediated cytotoxicity in mice bearing MSB sarcomas. J. Natl. Cancer Inst. 63: 479-483 (1979).
18. Nechay, B. R., Williams, B. J., Steinsland, O. S., and Hall, C. E. Increased vascular response to adrenergic stimulation in rats exposed to cadmium. J. Toxicol. Environ. Health 4: 559-567 (1978).
19. Bozelka, B. E., Burkholder, P. M., and Chang, L. W. Cadmium, a metallic inhibitor of antibody-mediated immunity in mice. Environ. Res. 17: 390-402 (1978).
20. Gamulin, S., Car, N., and Narancsik, P. Effect of cadmium on polyribosome structure and function in mouse liver. Experientia 53: 1144-1145 (1977).
21. Loose, L. D., Silkworth, J. B., and Warrington, D. Cadmium-induced depression of the respiratory burst in mouse pulmonary alveolar macrophages, peritoneal macrophages, and polymorphonuclear neutrophils. Biochem. Biophys. Res. Comm. 79: 326-332 (1977).
22. Fischer, A. B. Heavy metal toxicity in mammalian cell cultures. Zbl. Bakt, Hyg., I, Abt. Orig. B. 162: 77-84 (1976).
23. Gabridge, M. G., Bright, M. J., Nickerson, J. M., and Henderson, N. S. Development of an improved tracheal explant bioassay for the detection of the ciliary dyskinesia factor in cystic fibrosis serum. Pediat. Res. 13: 31-35 (1979).
24. Sanders, C. L., Hadley, J. G., Conklin, A. W., and Adee, R. R. Antagonism of cadmium-induced pulmonary toxicity by WR2721. Toxicol. Letters 2: 323-328 (1978).
25. Corradino, R. A. Cadmium inhibition of vitamin D-mediated responses in organ-cultured embryonic chick duodenum. Toxicol. Appl. Pharmacol. 48: 257-261 (1979).
26. Falchuk, K. H., Fawcett, D. W., and Vallee, B. L. Competitive antagonism of cadmium and zinc in the morphology and cell division of *Euglena gracilis*. J. Submicr. Cytol. 7: 139-152 (1975).
27. Elinder, C.-G., and Piscator, M. Cadmium and zinc relationships. Environ. Health Perspect. 25: 129-132 (1978).
28. Sugawara, N., and Sugawara, C. Effect of cadmium, in vitro and in vivo, on intestinal brush border ALPase and ATPase. Bull. Environ. Contam. Toxicol. 14: 653-656 (1975).
29. Nechay, B. R., and Saunders, J. P. Inhibition of renal adenosine triphosphatase by cadmium. J. Pharmacol. Exptl. Therap. 200: 623-629 (1977).
30. Allison, A. C. Subcellular pathology. In: The Cell in Medical Science, F. Beck and J. Lloyd, Eds., Academic Press, New York 1974, pp. 315-353.